

## FULL PAPER

## Design and synthesis of novel annulated thienopyrimidines as phosphodiesterase 5 (PDE5) inhibitors

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## Abstract

Novel cycloalkene-fused thienopyrimidine analogues with enhanced phosphodiesterase 5 (PDE5) inhibitory properties are presented. The structure of the reported scaffold was modulated through variation of the terminal cycloalkene ring size, as well as by varying the substituents at position 4 through the attachment of different groups including aniline, benzylamine, cyclohexylethylamine, methyl/acetyl/aryl piperazines, and aryl hydrazones. Compound **15Y** with a benzylamine substituent and cycloheptene as terminal ring showed the highest PDE5 inhibitory activity with an IC<sub>50</sub> value as low as 190 nM and with good selectivity versus PDE7 and PDE9.

## KEYWORDS

annulated thienopyrimidines, cycloalkene-fused thienopyrimidines, phosphodiesterase inhibitor, planarity



## 1 | INTRODUCTION

Phosphodiesterases (PDEs) are a group of enzymes that act by breaking down the secondary messengers cAMP (cyclic adenosine monophosphate) and cGMP (cyclic guanosine monophosphate) in cells to regulate signal transduction.<sup>[1]</sup> These secondary messengers are involved in diverse biological functions, including inflammation, skeletal muscle contraction, steroid hormone function, and memory. There are currently 11 PDE enzymes with multiple subtypes and splice variants yielding over

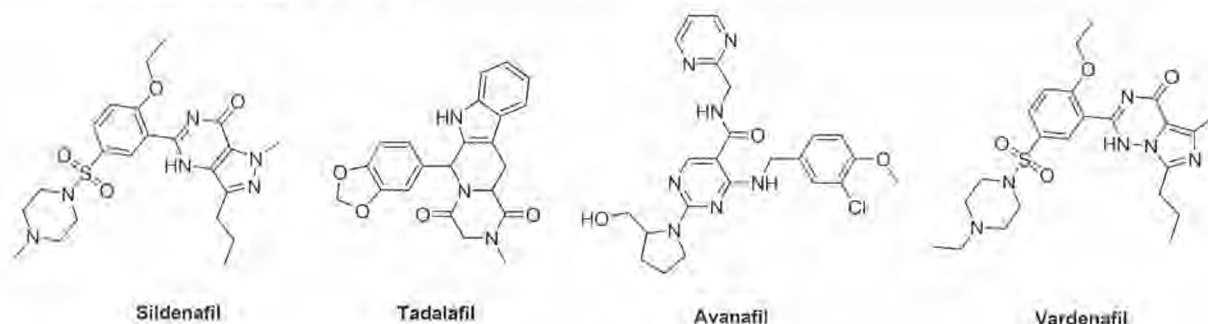
100 distinct isoforms. PDE isozyme families differ in structure, catalytic property, substrate specificity, regulatory properties, tissue localization, and inhibitor sensitivity.<sup>[2,3]</sup> The different gene products for PDEs are indicated by letters after the numbers and finally the Arabic numeral after the letter is indicative of the splice variant present.<sup>[4]</sup>

Four PDE5 inhibitors have been approved by the FDA as shown in Figure 1. PDE5 inhibitors are mainly indicated for the treatment of erectile dysfunction and pulmonary arterial hypertension. Although PDE5 inhibitors are widely used and generally well tolerated, these drugs can have serious side effects such as disturbance of vision. On the other hand, there are many emerging uses for PDE5 inhibitors including potential treatment of female sexual dysfunction, essential hypertension, benign prostatic hyperplasia, endothelial dysfunction, congestive heart failure, and Raynaud's phenomenon.<sup>[5,6]</sup> Additionally,

**Abbreviations:** cAMP, cyclic adenosine monophosphate; CC, column chromatography; cGMP, cyclic guanosine monophosphate; CREB, cAMP responsive element binding; N-terminal, amino terminal; PDE, phosphodiesterase; UV, ultraviolet.

Lina Y. El-Sharkawy and Rowaida A. El-Sakhawy contributed equally to the work.





**FIGURE 1** Structures of the approved PDE5 inhibitors

PDE5 inhibition was shown to play an important role in treatment of schizophrenia, improvement of cognitive function as well as improvement of hearing.<sup>[7,8]</sup> In the field of cancer therapy, PDE5 inhibitors showed the ability to reverse multiple drug resistance to several anticancer agents. Recent studies in a mouse model of colitis-induced colorectal cancer suggest that PDE5 inhibitors may provide chemopreventive benefits for individuals with inflammatory bowel disease who are at high risk of developing colorectal cancer.<sup>[9]</sup>

The chemical structure for PDE isozymes includes three domains: a variable regulatory domain, a highly conserved catalytic core, and the amino acid terminal showing isoform specificity. The N-terminus of the mRNA transcript is responsible for intracellular targeting domains, signalosomes, responsible for localization of PDEs to different organelles and membranes.<sup>[3,10]</sup>

Mammalian PDEs exist as homodimers, with the exception of PDE1 and PDE6, which occur as heterotetramers under physiological conditions. Biochemical observations showed dimer formation for both catalytic and regulatory domains. The catalytic domain, containing a deep hydrophobic pocket is folded into 16 helices. In the active site, the presence of glutamine residue is involved in recognition and selectivity to cyclic nucleotides. A histidine moiety is also important for catalysis as well as divalent zinc metal ions, showing co-ordination between conserved amino acids histidine and aspartate and magnesium occupying the M pocket.<sup>[11-13]</sup>

Glutamine, phenylalanine and either cyclic nucleotide substrate, product or inhibitor and a hydrophobic residue form the hydrophobic P clamp (part of the Q pocket). Glutamine and phenylalanine residues are responsible for anchorage of inhibitors in the pocket. Along with the M pocket, the active site also contains the S pocket (solvent-filled pocket). Sildenafil and tadalafil were co-crystallized with PDE5 (PDB 1UDU and 2H42) and showed common interactions with the enzyme, including: hydrogen bonding with Gln817 and  $\pi$ - $\pi$  stacking with Phe820.<sup>[11-15]</sup>

Many thienopyrimidines have been developed and evaluated for different pharmacological effects including anti-viral, anti-inflammatory, anti-microbial, anti-cancer activities and most importantly potential cGMP PDE inhibitors as well as cAMP PDE inhibitors.<sup>[16-23]</sup> In this work, we present a novel series of annulated thieno[2,3-*d*]-pyrimidine derivatives with a detailed study of the structure-activity relationship of this class of compounds as PDE5 inhibitors. A docking experiment is done to check the interactions of potent molecules with the protein.

## 2 | RESULTS AND DISCUSSION

### 2.1 | Chemistry

Thieno[2,3-*d*]pyrimidine derivatives have been reported as inhibitors of various PDE isozymes including PDE4, PDE5, PDE7, PDE9, and PDE10 (Figure 2).<sup>[19-23]</sup>

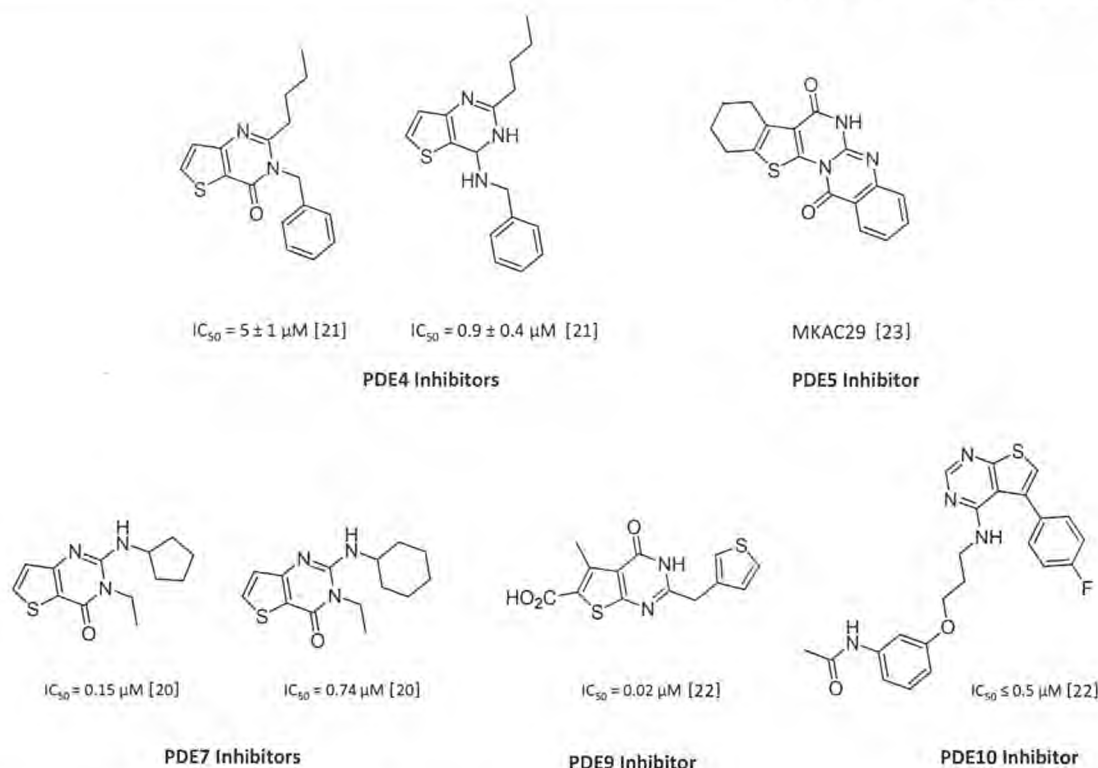
Thus, it is of considerable interest to design and synthesize novel thieno[2,3-*d*]pyrimidine derivatives for discovery of the scope and limitations of this class of compounds to inhibit PDE5. Our design strategy was aimed at synthesizing thieno[2,3-*d*]pyrimidines annulated to different rings of different sizes and lipophilicities, including cyclopentene and cycloheptene. Derivatization of this scaffold was examined through the attachment of different substituents to position 4 of the annulated thienopyrimidine. Substituents included unsubstituted or substituted aryl, alkyl, cycloalkyl that are separated from the main scaffold by varying spacer lengths from one atom, including an amino group, two atoms like the methylamino spacer, three atoms including an ethylamino, as well as a hydrazone spacer, and four atoms including a cyclized ethylenediamine moiety (i.e., piperazine).

One of the essential features required for PDE5 inhibition is the presence of a planar ring structure to interact with Phe820 by  $\pi$ - $\pi$  stacking and a hydrogen bonding interaction with Gln817. Binding pockets near the invariant glutamine residue differ in both shape and hydrophobicity which is why inhibitors show variable selectivity for PDE5.<sup>[12,13,24]</sup>

#### 2.1.1 | Synthesis

Two parallel sets of compounds were synthesized; one processes the thieno[2,3-*d*]pyrimidine fused to cyclopentene, while the second one has a cycloheptene ring. The synthesis of the starting 2-substituted aminothiophene was performed using the multicomponent single-pot Gewald reaction. Generally, the reaction involves an aldehyde or ketone, sulfur,  $\alpha$ -activated nitrile, and a base usually diethylamine or morpholine in the presence of ethanol or methanol as a solvent at temperature of 50°C.<sup>[25]</sup> Compounds **1A** and **1B** (Scheme 1) were synthesized using a mixture of cyclopentanone or cycloheptanone, respectively, ethyl cyanoacetate and sulfur in the presence of triethylamine in ethanol and left overnight. Reaction of compounds **1A** and **1B** with formamidine acetate was carried out according to the





**FIGURE 2** Structures of thienopyrimidines as PDE inhibitors

reported procedures, under reflux to give the cyclized intermediates, compounds **2A** and **2B**, respectively (Scheme 1).

Conversion of the hydroxyl group in compounds **2A** and **2B** to the chloro substituent in compounds **3A** and **3B**, respectively, was a crucial step to have an effective leaving group for further aromatic nucleophilic substitution to occur in the following step. In order to optimize the reaction conditions for chlorination several trials were carried out including the use of  $POCl_3$  only,  $POCl_3/TEA$  in a ratio 1:3. It is worth mentioning that chlorination did not succeed when only phosphoryl chloride was used. The mixture  $POCl_3/TEA$  in a ratio 1:3 was shown to be optimum. The reaction was left for 24 h at  $60^\circ C$ . Aromatic nucleophilic substitution reaction between the different amine derivatives with compounds **3A** and **3B** produced compounds **1X–15X**, **17X**, and **1Y–16Y**, respectively (Scheme 1).

Reaction of the chloro derivatives, compounds **3A** and **3B** with hydrazine monohydrate yielded compounds **4X** and **4Y**, respectively (Scheme 2). Compounds **18X–26X** and **18Y–26Y** (Scheme 2) were obtained by the reaction of different aryl aldehydes with compounds **4A** and **4B** to give the respective aryl hydrazone derivatives through an addition–elimination mechanism. The possibility of having one or two geometric isomers, *E* and *Z*, was possible. Some of the hydrazone derivatives were found to be *E/Z* mixtures. The  $^{13}C$  NMR in some of the compounds was confirmatory evidence since there is a doubling in the aliphatic and the aromatic carbons. However, other compounds were less likely to be mixture of isomers as indicated by their NMR spectra.

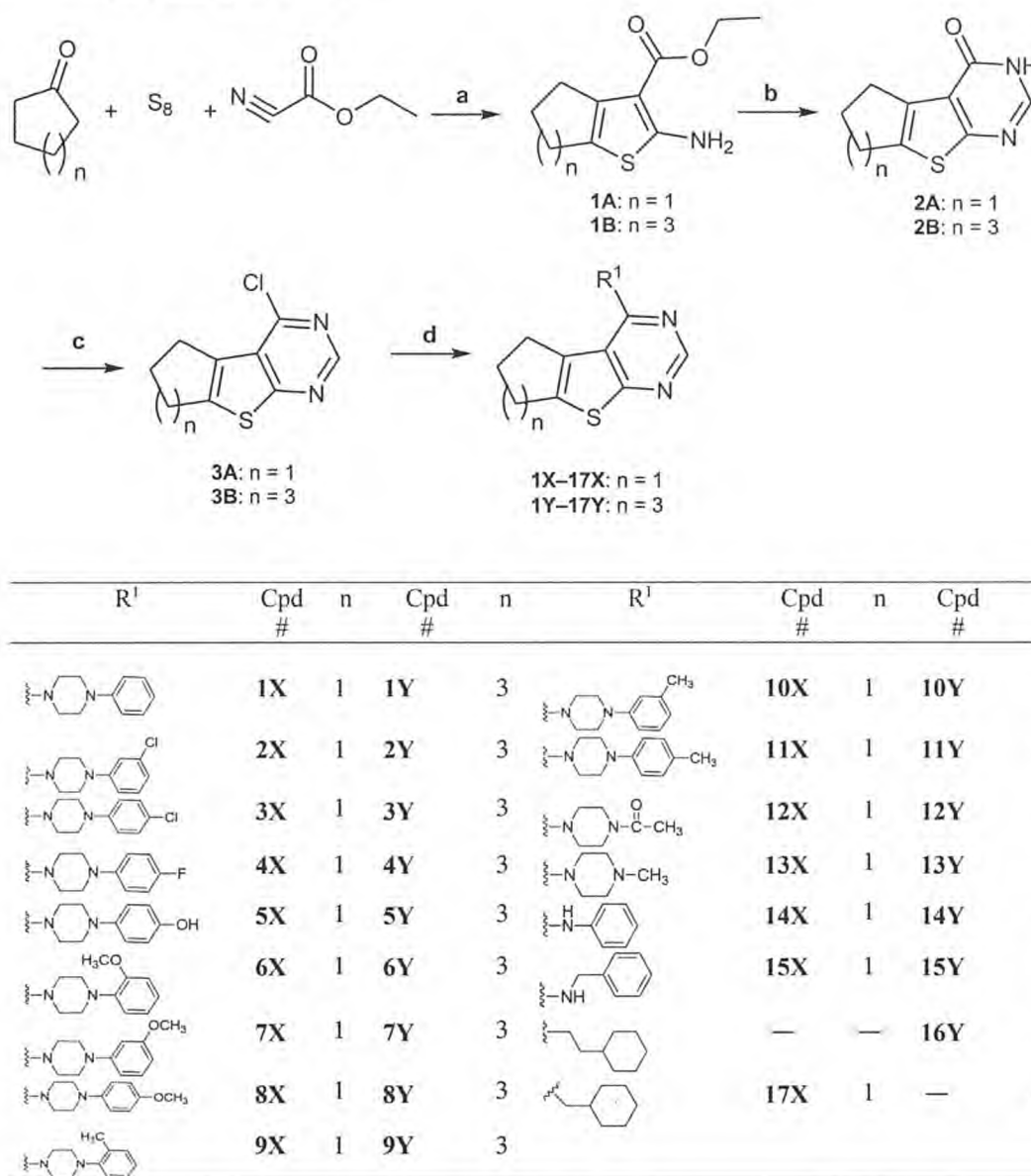
## 2.2 | Biological activity

### 2.2.1 | Structure–activity relationship of PDE5 inhibitory activity

The synthesized compounds were tested for PDE5 inhibition at  $25 \mu M$ ,  $IC_{50}$  was determined for compounds having a percentage inhibition of greater than 50%. Results are shown in Tables 1 and 2. According to the substitution type at position 4, the compounds synthesized can be divided into three main clusters; the piperazine derivatives, aryl, aralkyl as well as cycloalkyl amines and aryl hydrazones.

#### The 4-phenylpiperazine derivatives

Results showed that the presence of plain phenyl in compounds **1X** and **1Y** gave weak inhibition of less than 50% regardless of the terminal ring size being five-membered or seven-membered. Adding electron-withdrawing lipophilic halogens such as the *m*-chloro, *p*-chloro (compounds **2X–3X** and **2Y–3Y**), and *p*-fluoro (compounds **4X** and **4Y**) did not boost the activity where all of these derivatives showed percentage inhibition of less than 30%. On the other hand, the presence of the electron-donating methoxy in both *meta* and *para* positions (compounds **7X**, **7Y** and **8X**, **8Y**) generally gave more active compounds, where the % inhibition observed was between 52 and 68% with the best activity shown by the *m*-methoxyphenylpiperazine compound **7Y** ( $IC_{50} = 8.5 \mu M$ ). Conversely, shifting the methoxy group to the *ortho* position remarkably reduced the activity as shown in



**SCHEME 1** Reagents and conditions: (a) CH<sub>3</sub>CH<sub>2</sub>OH, Et<sub>3</sub>N, reflux 24 h, 100°C, (b) formamidine acetate, DMF, reflux 24 h, 80°C, (c) POCl<sub>3</sub>, Et<sub>3</sub>N, 70°C, 24 h, (d) amine derivative, EtOH, reflux 24 h

compounds **6X** and **6Y**. Demethylation of the *p*-methoxy in compounds **8X** and **8Y** to give a *p*-hydroxyl substituent in compounds **5X** and **5Y** maintained comparable potency. However, using the lipophilic electron donating methyl in the *meta* and *para* positions (compounds **10X**, **11X** and **10Y**, **11Y**) instead of the more polar methoxy group at similar positions failed to maintain the same level of potency indicating that the methoxy oxygen might play an important role via its +m effect and enriching the electron density of the aromatic system.

#### 4-Methyl/acetyl piperazines

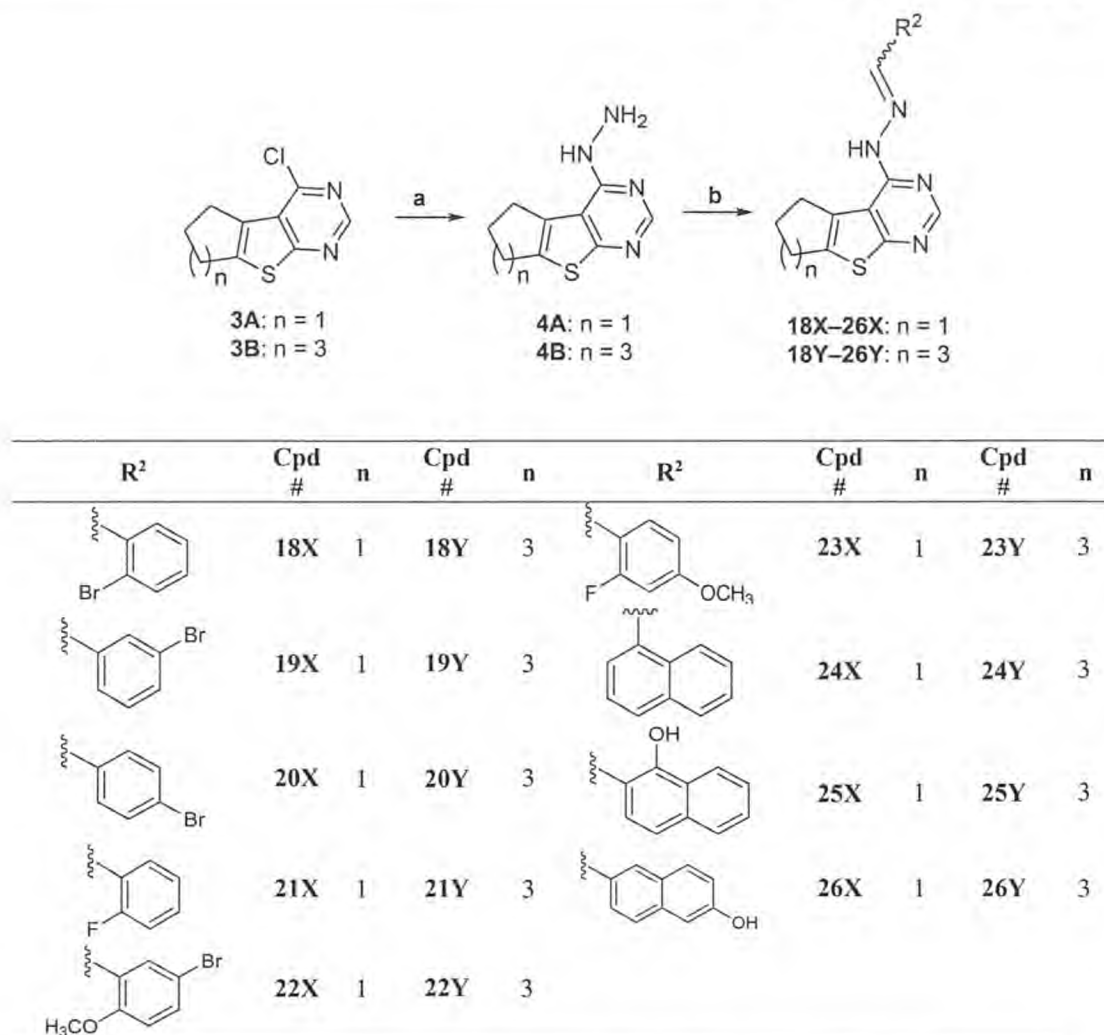
Both acetyl and methyl piperazines were tried at position 4 of the thienopyrimidine scaffold. Only the acetyl could maintain the marginal activity with % inhibition slightly greater than 50% at 25 μM as seen in

compounds **12X** and **12Y**, this might be due to additional polar interactions by the carbonyl oxygen.

#### Phenylamino/cycloalkylamino

Using the aniline moiety at position 4 (compounds **14X** and **14Y**) gave inhibition of 53 and 46%, respectively, at 25 μM with superior activity to the non-substituted phenyl piperazine derivatives (compounds **1X** and **1Y**), suggesting that the phenyl group in close proximity to position 4 is preferable for PDE5 inhibitory activity. Inserting a carbon spacer between the 4-amino group and the phenyl (i.e., using benzylamine) led to a significant increase in potency by more than 60-fold in the cyclopentene series and 130-fold in the cycloheptene series (compounds **15X** and **15Y**) to give the most two potent compounds in present series with IC<sub>50</sub> values of 0.42 and 0.19 μM, respectively.





**SCHEME 2** Reagents and conditions: (a) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O reflux 24 h, MeOH, reflux 2 h, (b) RCHO, EtOH, reflux 24 h

Employing the cyclohexylmethyl amine gave the saturated analog of compound **15X** which has similar activity (compound **17X**, IC<sub>50</sub> = 0.58 μM), suggesting that it is the effect of the hydrophobic interactions, rather than the π-mediated interactions, that predominantly improved the potency. However, increasing the spacer length between N4 and the cyclohexyl into two carbons reduced the potency five times. This was revealed by comparing compound **16Y** (IC<sub>50</sub> = 0.96 μM) with compound **15Y** (IC<sub>50</sub> = 0.19 μM), which might be due to the shift of the cyclohexyl from the optimum position for interaction with PDE5 pocket as well as the change in conformation of **16Y**. This can be shown in the energy minimized forms where the cyclohexylethyl is in the coplanar conformation to the cycloheptene-thienopyrimidine scaffold relative to the non-coplanar conformation of the benzyl in compound **15Y** with the cycloheptenethienopyrimidine scaffold, revealing the effect of the size of carbon spacer on the planarity of the molecule and its activity (Figure 3).

#### 4-Arylhydrazones

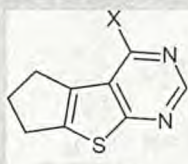
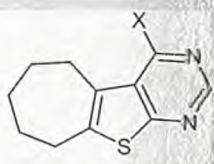
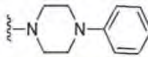
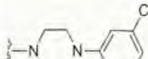
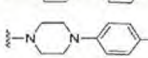
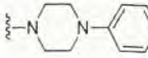
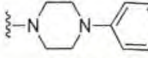
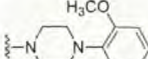
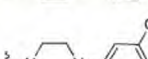
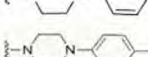
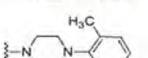

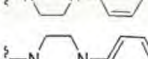
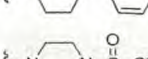
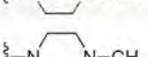

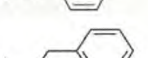
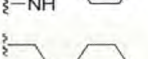
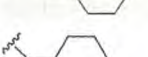
Several arylhydrazone moieties were connected to position 4 of the thienopyrimidine scaffold, three of the halo phenylhydrazones showed

% inhibition of greater than or equal to 50% at the screening concentration, namely compound **20X** (with 4-bromo substitution), **21X** (with 2-fluoro substitution), and **23X** (with 2-fluoro-4-methoxy substitution). Compounds **20X**, **21X**, and **23X** showed IC<sub>50</sub> values of 25, 19.3, and 21.8 μM, respectively. This modest activity indicated that the rigid and long hydrazone spacer adopted in the 4-arylhydrazone derivatives was deleterious to the activity. Additionally, it was also observed in this group of compounds that only the cyclopentene analogs but not the respective cycloheptene compounds exhibited significant activity. Furthermore, all of the bulkier naphthylhydrazone analogues were totally inactive whether they belong to the cyclopentene or the cycloheptene derivatives (compounds **24X–26X** and **24Y–26Y**).

#### Cyclopentene versus cycloheptene derivatives

By comparing the % PDE5 inhibition of the piperazine derivatives (Table 1), it can be seen that many cyclopentene derivatives showed similar inhibition to the respective cycloheptene analogues, giving indication that the terminal ring size and its lipophilicity are not major determinants for activity among these compounds. However, by comparing the most two potent compounds, the benzylamine derivatives

**TABLE 1** Inhibition of PDE5 by compounds 1X-17X and 1Y-17Y

|   |      | <br>1X-17X |                                  | <br>1Y-17Y |                                 |                                  |
|---|------|---|----------------------------------|--|---------------------------------|----------------------------------|
| X   | Cpd# | PDE5 % inhibition at 25 $\mu$ M   | PDE5 IC <sub>50</sub> ( $\mu$ M) | Cpd#   | PDE5 % inhibition at 25 $\mu$ M | PDE5 IC <sub>50</sub> ( $\mu$ M) |
|    | 1X   | 23  | ND                               | 1Y   | 39                              | ND                               |
|    | 2X   | 28  | ND                               | 2Y   | 18                              | ND                               |
|    | 3X   | 28  | ND                               | 3Y   | 0                               | ND                               |
|    | 4X   | 30  | ND                               | 4Y   | 27                              | ND                               |
|    | 5X   | 47  | ND                               | 5Y   | 77                              | 1.2                              |
|    | 6X   | 27  | ND                               | 6Y   | 0                               | ND                               |
|    | 7X   | 64  | 12.6                             | 7Y   | 52                              | 15                               |
|   | 8X   | 68  | 21                               | 8Y   | 55                              | 14.5                             |
|  | 9X   | 22  | ND                               | 9Y   | 40                              | ND                               |
|  | 10X  | 25  | ND                               | 10Y  | 19                              | ND                               |
|  | 11X  | 10  | ND                               | 11Y  | 39                              | ND                               |
|  | 12X  | 57  | 22                               | 12Y  | 55                              | 11                               |
|  | 13X  | 40  | ND                               | 13Y  | 31                              | ND                               |
|  | 14X  | 53  | 25                               | 14Y  | 45                              | ND                               |
|  | 15X  | 98  | 0.42                             | 15Y  | 86                              | 0.19                             |
|  | 16X  | -   | -                                | 16Y  | 17                              | 156                              |
|  | 17X  | 100   | 0.58                             | 17Y  | -                               | ND                               |

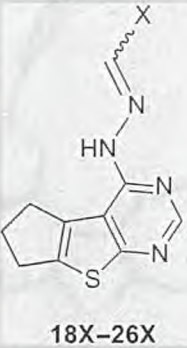
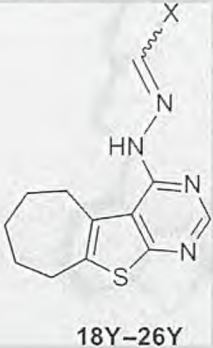

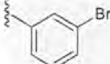
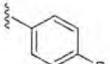

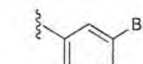
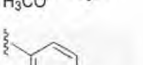

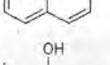
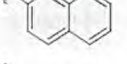
(15X and 15Y), the cycloheptene derivative (15Y) showed more than twofold potency of the corresponding cyclopentene congener, which could be due to a better positioning of the benzyl group in its critical interaction with the pocket as an effect of the expanded terminal ring (Figure 3). On the contrary, the expanded cycloheptene ring decreased the activity of the hydrazone analogues (Table 2) as described above.

## 2.2.2 | Docking and molecular modeling

Docking was performed on silodosin and one of the most active compounds, compound 15Y, using AutoDock (version 2016.08) on the PDE5 crystal structure 2HP2. Comparing the 2D interaction maps of silodosin in the pocket (Figure 4) and compound



**TABLE 2** Inhibition of PDE5 by the arylhydrazones

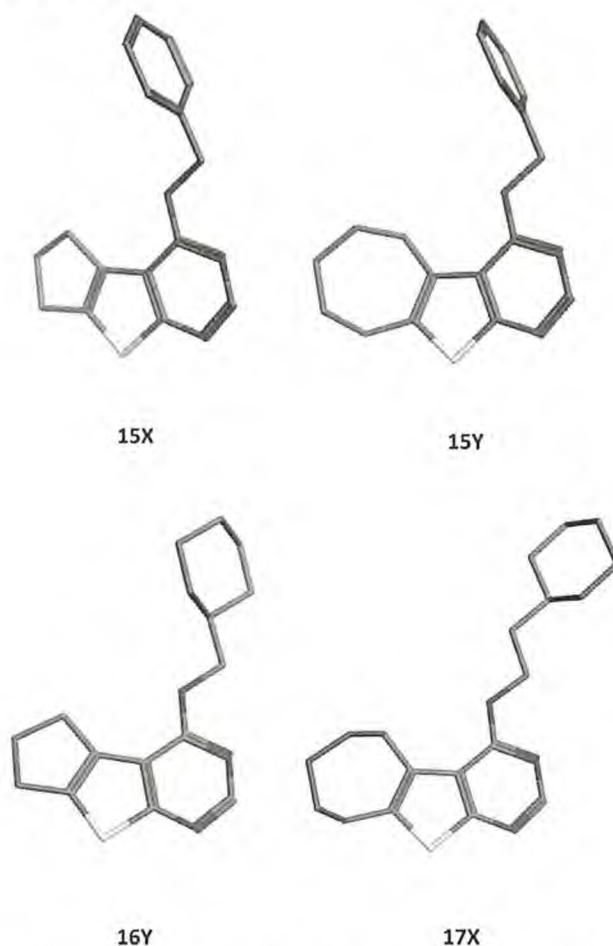
| <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>18X–26X</p> </div> <div style="text-align: center;">  <p>18Y–26Y</p> </div> </div> |      |                                 |                                  |      |                                 |                                  |
|--|------|---------------------------------|----------------------------------|------|---------------------------------|----------------------------------|
| X  | Cpd# | PDE5 % inhibition at 25 $\mu$ M | PDE5 IC <sub>50</sub> ( $\mu$ M) | Cpd# | PDE5 % inhibition at 25 $\mu$ M | PDE5 IC <sub>50</sub> ( $\mu$ M) |
|   | 18X  | 25                              | ND                               | 18Y  | 0                               | ND                               |
|   | 19X  | 40                              | ND                               | 19Y  | 4                               | ND                               |
|   | 20X  | 50                              | 25                               | 20Y  | 15                              | ND                               |
|   | 21X  | 60                              | 19.3                             | 21Y  | 28                              | ND                               |
|    | 22X  | 29                              | ND                               | 22Y  | 19                              | ND                               |
|   | 23X  | 55                              | 21.8                             | 23Y  | 23                              | ND                               |
|   | 24X  | 0                               | ND                               | 24Y  | 2                               | ND                               |
|   | 25X  | 0                               | ND                               | 25Y  | 0                               | ND                               |
|   | 26X  | 12                              | ND                               | 26Y  | 1                               | ND                               |

**15Y** (Figure 4B), compound **15Y** maintained some of the critical interactions made by sildenafil, importantly hydrogen bonding with the invariant glutamine residue Gln817 through the NH at position 4 of the cycloheptenethienopyrimidine scaffold and secondly  $\pi$ - $\pi$  stacking interaction was observed for compound **15Y** with Phe820 similar to that made with sildenafil. The energy minimized form for compound **15Y** inside the PDE5 pocket indicates that the phenyl ring is present in a non-coplanar conformation to the cycloheptenethienopyrimidine scaffold as can be seen in Figure 5. The non-coplanarity appears to be required for interaction of the phenyl ring inside the pocket. Some of the hydrophobic residues in the pocket include Phe786, Leu804, and Ile813 (Figure 4). Figure 6 shows similar interactions made by

compound **15Y** and sildenafil with essential PDE5 residues upon overlay of the two structures.

### 2.2.3 | Selectivity for PDE5 against PDE7 and PDE9 inhibition

The most active compounds for PDE5 inhibition including compounds **15X**, **15Y**, **16Y**, and **17X** with percentages of inhibition at 98, 88, 87, and 100%, respectively, showed weak activity of less than 33% for both PDE7 and PDE9 inhibition at 25  $\mu$ M. For % PDE7 inhibition, percentages were 28 and 22% for compounds **15X** and **15Y**, respectively, and for **16Y** and **17X** it was 6 and 28%, respectively. For PDE9, the % inhibition values were 23 and 11%



**FIGURE 3** Energy minimized forms of the most active compounds on PDE5 showing the effect of ring size on the planarity for compounds 15X and 15Y and of variation of carbon spacer on the planarity of compounds 16X and 17Y

for compounds 15X and 15Y, respectively, and 3 and 32% inhibition for 16Y and 17X, respectively. Thus, the most active compounds for PDE5 inhibition showed greater selectivity toward PDE5 compared to PDEs 7 and 9.

### 3 | CONCLUSION

Compound 15Y ( $IC_{50} = 190$  nM) can serve as a promising lead compound for further optimization as PDE5 inhibitor through using different substituted benzyl derivatives to reach the single digit nM range, this is encouraged by the advantageous selectivity over PDE7 and PDE9.

## 4 | EXPERIMENTAL

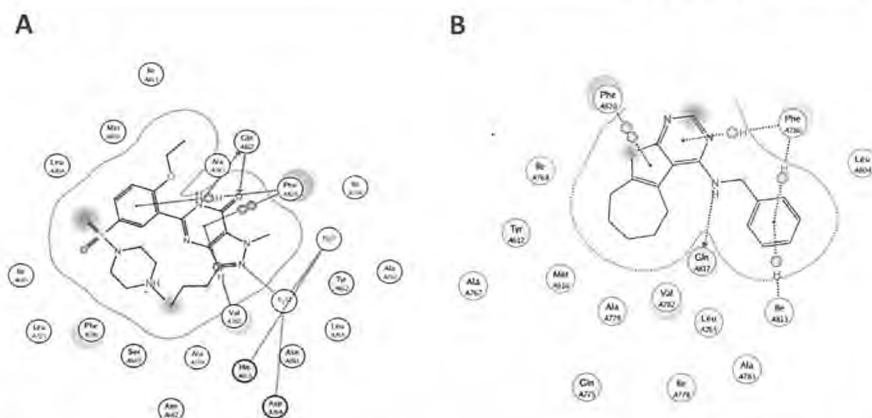
### 4.1 | Chemistry

#### 4.1.1 | General

Solvents and reagents were obtained from commercial suppliers and were used without further purification. All solvents used were of pure analytical grade. Column chromatography was carried out using silica

gel 60 (0.063–0.200 mm). Reaction progress was monitored by TLC using fluorescent pre-coated silica gel plates and detection of the components was made by short UV light ( $\lambda = 254$  nm).  $^1H$  NMR and  $^{13}C$  NMR spectra were recorded using a Bruker Fourier 300, Varian Mercury 400 or Bruker DRX 500.  $^1H$  shifts are referenced to the residual protonated solvent signals  $\delta$  2.50 for dimethylsulfoxide ( $DMSO-d_6$ ) and  $\delta$  7.2 for chloroform- $d$  ( $CDCl_3$ ).  $^{13}C$  shifts are referenced to the deuterated solvent signal  $\delta$  39.5 for  $DMSO-d_6$  and  $\delta$  77.0 for  $CDCl_3$ . Coupling constants ( $J$ ) are given in hertz (Hz). Multiplicities are abbreviated as s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet; dd: doublet of doublet; dt: doublet of triplet; brs: broad singlet. Mass spectrometric analysis (HPLC-ESI-MS) was performed on a TSQ quantum (Thermo Electron Corporation) instrument equipped with an ESI source and a triple quadrupole mass detector (Thermo Finnigan, San Jose, CA). The MS detection was carried out at a spray voltage of 4.2 kV, a nitrogen sheath gas pressure of  $4.0 \times 10^5$  Pa, an auxiliary gas pressure of  $1.0 \times 10^5$  Pa, a capillary temperature of 400°C, capillary voltage of 35 V, and source CID of 10 V. All samples were injected by autosampler (Surveyor, Thermo Finnigan) with an injection volume of 10  $\mu$ L. A RP C18 NUCLEODUR 100-3 (125 mm  $\times$  3 mm) column (Macherey-Nagel) was used as





**FIGURE 4** (A) 2D image of the detailed mode view showing the docked pose of sildenafil with human PDE5. (B) 2D image of the detailed mode view showing the docked pose of compound **15** with human PDE5 enzyme

stationary phase. The solvent system consisted of water containing 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). HPLC Experimental 63 method: flow rate 400  $\mu$ L/min. The percentage of B started at an initial of 5%, was increased up to 100% during 16 min, kept at 100% for 2 min, and flushed back to 5% in 2 min. All masses were reported as those of the protonated parent ions. The purities of the tested compounds were determined by HPLC coupled with mass spectrometry and were higher than 95% purity. Measurements for melting point were not corrected and were recorded using capillary Buchi B-540 melting point apparatus.

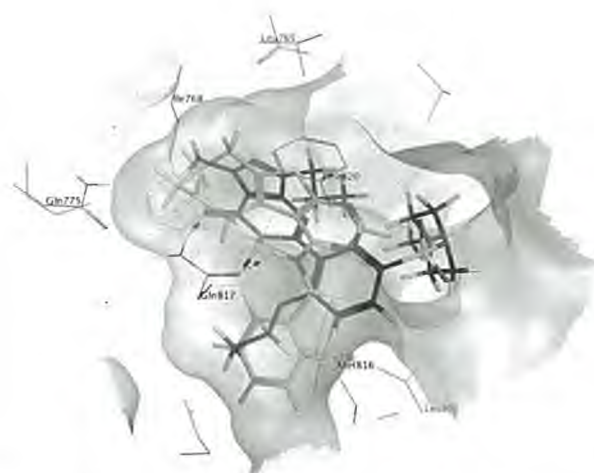
The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

#### 4.1.2 | General procedure for Gewald reaction for the synthesis of compounds **1A** and **1B**

A 100 mL round bottom flask was charged with cyclopentanone or cycloheptanone (10 mmol), sulfur (0.32 g, 10 mmol), ethylcyano acetate (1.31 g, 4 mmol), morpholine (5 mL), and ethanol (10 mL). The reaction solution was stirred overnight at temperature not exceeding 65°C. The solvent was removed under reduced pressure, then brine was added and extraction was done using methylene chloride (3  $\times$  50 mL). The collected organic layers were dried over anhydrous  $MgSO_4$ , evaporated under reduced pressure, and the residue was purified using column chromatography to yield the desired product compound **1A** as a brown powder, 92% yield, mp of 91–92°C and compound **1B** as a yellow solid, 63% yield, mp of 88–89°C.<sup>[27,28]</sup>



**FIGURE 5** Detailed mode view of **15Y** in PDE5 pocket showing the non-coplanarity between the phenyl ring and the cycloheptenethienopyrimidine scaffold (PDB: 2H42). PDE5 binding site—red color: hydrophilic region, blue color: hydrophobic region, white color: solvent exposed region



**FIGURE 6** Graphical representation of the binding modes of sildenafil (brown) and compound **15Y** (gold) in the pocket of human PDE5 enzyme (PDB: 2H42). PDE5 binding site—red color: hydrophilic region, blue color: hydrophobic region, white color: solvent exposed region



#### 4.1.3 | General procedure for thieno[2,3-*d*]pyrimidin-4-ones synthesis of compounds 2A and 2B

Compound **1A** or **1B** (56.7 mmol) and formamidine acetate (8.85 g, 85.0 mmol) in DMF (100 mL) were heated at 80°C for 3 days. The reaction mixture was cooled, DMF removed in vacuum, and the solid obtained was washed thoroughly with water, extracted with ethyl acetate, and the crude product was purified by silica gel flash CC to give the desired products; **2A**, white solid: yield 40%, mp 242–244°C and **2B**, light brown solid: yield 59%, mp 118–220°C.<sup>[28,29]</sup>

#### 4.1.4 | General procedure for chlorination to synthesize compounds 3A and 3B

To a cooled mixture of POCl<sub>3</sub> (3 mL) and triethylamine (9 mL) was added compound **2A** or **2B** (4.54 mmol). The mixture was then heated at 70°C for 24 h. Ice water was poured into the mixture. The resulting mixture was extracted with ethyl acetate, the organic layer was separated, dried over MgSO<sub>4</sub>, and concentrated in vacuum to obtain the desired product that was used in the next step without further purification. Compound **3A** gave a yellow powder: yield 45%; mp 130–132°C, while compound **3B** gave a dark brown solid: yield 95%; mp 68–71°C.<sup>[28]</sup>

#### 4.1.5 | General procedure for hydrazination to synthesize compounds 4A and 4B

A mixture of the chloro derivative **3A** or **3B** (2 mmol) and hydrazine monohydrate (5 equiv.) was dissolved in (10 mL) methanol. The reaction mixture was kept under reflux for 3 h. After completion of the reaction (monitored by TLC), the precipitate formed was purely obtained by vacuum filtration and then washed with diethyl ether without any further purification. Compound **4A** gave a yellow powder: yield 86.8%, mp 223–225°C whereas compound **4B** gave a reddish brown solid: yield 78%; mp 145–147°C.<sup>[30]</sup>

#### 4.1.6 | General procedures for synthesis of *N*-(substituted)-cyclopentenethienopyrimidine-4-amine and *N*-(substituted)-cycloheptenethienopyrimidine-4-amine

A mixture of compound **3A** or **3B** (45 mg, 0.2 mmol) and the different amines (2.0 equiv, 0.4 mmol) in ethanol (3 mL) was refluxed for 24 h. The reaction mixture was concentrated in vacuum and the residue was partitioned between water and dichloromethane or ethyl acetate. The organic layers were dried over MgSO<sub>4</sub> and concentrated in vacuum. The crude product was purified by silica gel flash column chromatography to obtain compounds **1X–15X**, **17X** and **1Y–16Y** from **3A** and **3B**, respectively.<sup>[28]</sup>

##### 4-(4-Phenylpiperazine-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]-thieno[2,3-*d*]pyrimidine (**1X**)

The title compound was prepared by reaction of compound **3A** and 4-phenylpiperazine according to the general procedure. The product

was purified by CC (hexane/EtOAc, 85:15) to give orange crystals: yield 80%; mp 157–159°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.50 (s, 1H), 7.33–7.27 (m, 2H), 7.00 (t, *J* = 1.5 Hz, 1H), 6.99–6.97 (m, 1H), 6.91 (tt, *J* = 7.4, 1.0 Hz, 1H), 3.80–3.64 (m, 4H), 3.34–3.44 (m, 4H), 3.12–3.06 (m, 2H), 3.05–2.99 (m, 2H), 2.51–2.43 (m, 2H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 173.43, 160.73, 151.69, 151.28, 140.23, 135.75, 129.35, 120.38, 117.55, 116.49, 49.44, 49.41, 31.85, 29.99, 28.36; MS (ESI) *m/z* = 337.18 (M+H)<sup>+</sup>.

##### 4-(4-Phenylpiperazine-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (**1Y**)

The title compound was prepared by reaction of compound **3B** and 4-phenylpiperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 8:1) to give light brown crystals: yield 63%; mp 174.3–176.2°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.47 (s, 1H), 7.27–7.17 (m, 2H), 6.92 (dd, *J* = 8.7, 0.9 Hz, 2H), 6.87–6.80 (m, 1H), 3.48 (s, 4H), 3.29 (s, 4H), 3.07–3.00 (m, 2H), 2.86 (m, 2H), 1.92–1.82 (m, 2H), 1.74–1.56 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 161.84, 151.24, 151.20, 144.8, 139.43, 132.08, 129.24, 121.15, 120.26, 116.36, 50.25, 48.99, 32.68, 30.46, 28.74, 27.54, 27.33; MS (ESI) *m/z* = 364.17 (M+H)<sup>+</sup>.

##### 4-(4-(*m*-Chlorophenyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (**2X**)

The title compound was prepared by reaction of compound **3A** and 4-(*m*-chlorophenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 80:20) to give beige oil: yield 3.4%; mp 157–159°C; <sup>1</sup>H NMR (500 MHz, MeOD) δ 8.41 (s, 1H), 7.22 (t, *J* = 8.1 Hz, 1H), 7.01 (t, *J* = 2.2 Hz, 1H), 6.96–6.93 (m, 1H), 6.83 (ddd, *J* = 7.9, 1.9, 0.8 Hz, 1H), 3.80–3.73 (m, 4H), 3.36 (dd, *J* = 9.6, 4.6 Hz, 4H), 3.17–3.13 (m, 2H), 3.05 (dd, *J* = 10.4, 4.2 Hz, 2H), 2.50 (dt, *J* = 14.3, 7.3 Hz, 2H); <sup>13</sup>C NMR (126 MHz, MeOD) δ 173.71, 161.84, 153.94, 152.21, 141.46, 137.47, 136.01, 131.30, 120.54, 118.44, 116.98, 115.49, 50.18, 49.87, 32.79, 30.53, 29.33; MS (ESI) *m/z* = 371.14 (M+H)<sup>+</sup>.

##### 4-(4-(*m*-Chlorophenyl)piperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (**2Y**)

The title compound was prepared by reaction of compound **3B** and 4-(*m*-chlorophenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 5:1) to give a brown solid: yield 5.9%; mp 134.8–136.1°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.46 (s, 1H), 7.13 (t, *J* = 8.1 Hz, 1H), 6.86 (s, 1H), 6.78 (d, *J* = 8.1 Hz, 2H), 3.46 (s, 4H), 3.29 (s, 4H), 3.03 (m, 2H), 2.89–2.83 (m, 2H), 1.91–1.82 (m, 2H), δ 1.71–1.58 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 166.60, 161.73, 152.25, 151.21, 139.63, 135.07, 131.98, 130.14, 121.19, 119.89, 116.12, 114.27, δ 50.06, 48.52, 32.65, 30.46, 29.71, 28.72, 27.30; MS (ESI) *m/z* = 379.21 (M+H)<sup>+</sup>.

##### 4-(4-(*p*-Chlorophenyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (**3X**)

The title compound was prepared by reaction of compound **3A** and 4-(*p*-chlorophenyl)piperazine according to a general procedure. The product was purified by CC (hexane/EtOAc, 80:20) to give yellow



crystals; yield 94%; mp 172–174°C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.50 (s, 1H), 7.25–7.21 (m, 2H), 6.91–6.87 (m, 2H), 3.74–3.70 (m, 4H), 3.33–3.29 (m, 4H), 3.10–3.06 (m, 2H), 3.05–3.00 (m, 2H), 2.50–2.43 (m, 2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  173.47, 160.67, 151.67, 149.90, 140.40, 135.66, 129.20, 125.24, 117.68, 117.58, 49.42, 49.30, 31.82, 30.00, 28.36; MS (ESI)  $m/z$  = 371.05 (M+H) $^+$ .

**4-(4-(*p*-Chlorophenyl)piperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (3Y)**

The title compound was prepared by reaction of compound **3B** and 4-(*p*-chlorophenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 8:1) to give an off-white solid; yield 48%; mp 168.8–171°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.47 (s, 1H), 7.18–7.14 (m, 2H), 6.84–6.80 (m, 2H), 3.46 (s, 4H), 3.24 (s, 4H), 3.06–3.00 (m, 2H), 2.86 (m, 2H), 1.92–1.83 (m, 2H), 1.69–1.59 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.55, 161.73, 151.22, 149.80, 139.57, 131.99, 129.07, 125.12, 121.16, 117.56, 50.10, 48.97, 32.66, 30.46, 28.72, 27.54, 27.30; MS (ESI)  $m/z$  = 379.21 (M+H) $^+$ .

**4-(4-(*p*-Fluorophenyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (4X)**

The title compound was prepared by reaction of compound **3A** and 4-(*p*-fluorophenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 85:15) to give yellow crystals; yield 40%; mp 142–144°C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.44 (s, 1H), 7.11–7.04 (m, 2H), 7.04–6.98 (m, 2H), 3.69–3.64 (m, 4H), 3.26–3.22 (m, 4H), 3.07 (t,  $J$  = 7.1 Hz, 2H), 2.99 (t,  $J$  = 7.3 Hz, 2H), 2.43–2.35 (m, 2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ )  $\delta$  172.37, 159.78, 156.22 (d,  $J_{\text{CF}}$  = 236.0 Hz), 151.34, 147.76, 139.03, 135.82, 117.47 (d,  $J_{\text{CF}}$  = 7.6 Hz), 116.29, 115.34 (d,  $J_{\text{CF}}$  = 21.9 Hz), 49.01, 48.57, 31.29, 29.35, 27.75; MS (ESI)  $m/z$  = 355.18 (M+H) $^+$ .

**4-(4-(*p*-Fluorophenyl)piperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (4Y)**

The title compound was prepared by reaction of compound **3B** and 4-(*p*-fluorophenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 6:1) to give a white solid; yield 59.2%; mp 168.4–170.2°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.47 (s, 1H), 6.96–6.91 (m, 2H), 6.89–6.84 (m, 2H), 3.48 (s, 4H), 3.20 (s, 4H), 3.06–3.01 (m, 2H), 2.89–2.80 (m, 2H), 1.94–1.82 (m, 2H), 1.69–1.57 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.53, 161.77, 157.46 (d,  $J_{\text{CF}}$  = 239.5 Hz), 151.23, 147.82, 139.48, 132.03, 121.13, 118.18 (d,  $J_{\text{CF}}$  = 7.7 Hz), 115.65 (d,  $J_{\text{CF}}$  = 22.1 Hz), 50.25, 49.96, 32.67, 30.46, 28.74, 27.54, 27.31; MS (ESI)  $m/z$  = 383.18 (M+H) $^+$ .

**4-(4-(6,7-Dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)piperazin-1-yl)phenol (5X)**

The title compound was prepared by reaction of compound **3A** and 4-(*p*-hydroxyphenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 60:40) to give brown powder; yield 35.82%; mp 242–244°C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.89 (s, 1H), 8.43 (s, 1H), 6.88–6.81 (m, 2H), 6.70–6.64 (m, 2H), 3.68–

3.62 (m, 4H), 3.14–3.09 (m, 4H), 3.06 (t,  $J$  = 7.0 Hz, 2H), 2.98 (t,  $J$  = 7.3 Hz, 2H), 2.41–2.35 (m, 2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ )  $\delta$  172.34, 159.83, 151.30, 151.25, 143.98, 138.89, 135.94, 118.18, 116.27, 115.50, 50.12, 48.79, 31.32, 29.35, 27.76; MS (ESI)  $m/z$  = 353.20 (M+H) $^+$ .

**4-(4-(6,7,8,9-Tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)piperazin-1-yl)phenol (5Y)**

The title compound was prepared by reaction of compound **3B** and 4-(*p*-hydroxyphenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 2:1) to give a dark brown solid; yield 76.2%; mp 230–233.6°C;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.90 (s, 1H), 8.48 (s, 1H), 6.84 (d,  $J$  = 8.8 Hz, 2H), 6.67 (d,  $J$  = 8.7 Hz, 2H), 3.10 (m, 2H), 2.90 (d,  $J$  = 4.7 Hz, 2H), 1.88 (m, 2H), 1.64 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  165.37, 161.18, 151.15, 150.92, 143.84, 138.37, 132.12, 120.02, 118.06, 115.41, 49.87, 49.62, 31.85, 29.49, 28.06, 26.80, 26.79; MS (ESI)  $m/z$  = 381.17 (M+H) $^+$ .

**4-(4-(*o*-Methoxyphenyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (6X)**

The title compound was prepared by reaction of compound **3A** and 4-(*o*-methoxyphenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 70:30) to give beige crystals; yield 69.2%; mp 140–142°C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.43 (s, 1H), 7.00–6.93 (m, 3H), 6.89 (ddd,  $J$  = 7.9, 6.5, 2.2 Hz, 1H), 3.80 (s, 3H), 3.68 (dd,  $J$  = 11.8, 7.0 Hz, 4H), 3.11 (dd,  $J$  = 9.9, 5.2 Hz, 4H), 3.07 (t,  $J$  = 7.0 Hz, 2H), 2.98 (t,  $J$  = 7.3 Hz, 2H), 2.38 (q,  $J$  = 7.4 Hz, 2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ )  $\delta$  172.35, 159.76, 152.02, 151.30, 140.86, 138.73, 136.02, 122.82, 120.85, 118.24, 116.16, 111.92, 55.39, 50.06, 48.86, 31.38, 29.36, 27.78; MS (ESI)  $m/z$  = 367.21 (M+H) $^+$ .

**4-(4-(*o*-Methoxyphenyl)piperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (6Y)**

The title compound was prepared by reaction of compound **3B** and 4-(*o*-methoxyphenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 2:1) to give a white solid; yield 48.4%; mp 180.9–182.1°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.46 (d,  $J$  = 1.5 Hz, 1H), 7.00–6.79 (m, 4H), 3.53 (s, 4H), 3.17 (s, 4H), 3.07 (m, 2H), 2.88–2.80 (m, 2H), 1.86 (m, 2H), 1.64 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.50, 161.81, 160.68, 152.58, 151.23, 139.45, 132.06, 129.92, 121.15, 109.10, 104.94, 102.88, 55.26, 50.18, 48.89, 32.67, 30.46, 28.73, 27.53, 27.32; MS (ESI)  $m/z$  = 331.17 (M+H) $^+$ .

**4-(4-(*m*-Methoxyphenyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (7X)**

The title compound was prepared by reaction of compound **3A** and 4-(*m*-methoxyphenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 80:20) to give beige powder; yield 63%; mp 112–114°C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.50 (s, 1H), 7.23–7.19 (m, 1H), 6.60 (ddd,  $J$  = 8.3, 2.4, 0.7 Hz, 1H), 6.52 (t,  $J$  = 2.3 Hz, 1H), 6.46 (ddd,  $J$  = 8.2, 2.4, 0.7 Hz, 1H), 3.81 (s, 3H), 3.75–3.69 (m, 4H), 3.43–3.30 (m, 4H), 3.11–3.06 (m, 2H), 3.05–3.00 (m, 2H), 2.50–2.43 (m, 2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  173.18, 160.53,



160.44, 152.40, 151.42, 140.00, 135.48, 129.79, 117.28, 108.95, 104.81, 102.70, 55.12, 49.08, 31.60, 29.74, 28.11; MS (ESI)  $m/z = 366.95$  (M+H)<sup>+</sup>.

**4-(4-(*m*-Methoxyphenyl)piperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (7Y)**

The title compound was prepared by reaction of compound **3B** and 4-(*m*-methoxyphenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 4:1) to give an off-white solid; yield 54.4%; mp 118–119°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.47 (s, 1H), 7.20–7.09 (m, 1H), 6.52 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.45 (t, *J* = 2.1 Hz, 1H), 6.39 (dd, *J* = 8.1, 2.2 Hz, 1H), 3.73 (s, 3H), 3.46 (s, 4H), 3.28 (s, 4H), 3.06–3.00 (m, 2H), 2.84 (m, 2H), 1.92–1.81 (m, 2H), 1.65 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 166.50, 161.81, 160.68, 152.58, 151.23, 139.45, 132.06, 129.92, 121.15, 109.10, 104.94, 102.88, 55.26, 50.18, 48.89, 32.67, 30.46, 28.73, 27.53, 27.32; MS (ESI)  $m/z = 395.17$  (M+H)<sup>+</sup>.

**4-(4-(*p*-Methoxyphenyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (8X)**

The title compound was prepared by reaction of compound **3A** and 4-(*p*-methoxyphenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 70:30) to give beige powder; yield 74.9%; mp 151–155°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.49 (s, 1H), 7.00–7.1 (m, 2H), 6.88–6.84 (m, 2H), 3.78 (d, *J* = 3.6 Hz, 4H), 3.76 (s, 3H), 3.32–3.17 (m, 4H), 3.16–3.04 (m, 2H), 3.02 (t, *J* = 7.3 Hz, 2H), 2.55–2.38 (m, 2H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 173.46, 160.68, 151.68, 140.21, 135.78, 118.85, 117.51, 114.72, 55.73, 51.08, 49.40, 31.88, 29.99, 28.37; MS (ESI)  $m/z = 367.22$  (M+H)<sup>+</sup>.

**4-(4-(*p*-Methoxyphenyl)piperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (8Y)**

The title compound was prepared by reaction of compound **3B** and 4-(*p*-methoxyphenyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 4:1) to give yellow powder; yield 54.4%; mp 131.2–132°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.47 (s, 1H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 2H), 3.71 (s, 3H), 3.48 (s, 4H), 3.17 (s, 4H), 3.06–2.99 (m, 2H), 2.88–2.81 (m, 2H), 1.86 (m, 2H), 1.72–1.56 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 166.47, 161.83, 154.17, 151.24, 145.52, 139.33, 132.10, 121.10, 118.49, 114.55, 55.62, 50.42, 50.35, 32.68, 30.46, 28.76, 27.54, 27.33; MS (ESI)  $m/z = 395.17$  (M+H)<sup>+</sup>.

**4-(4-(*o*-Tolyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (9X)**

The title compound was prepared by reaction of compound **3A** and 4-(*o*-tolyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 80:20) to give white crystals; yield 72.89%; mp 124–126°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.50 (s, 1H), 7.23–7.17 (m, 2H), 7.07 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.02 (td, *J* = 7.4, 1.2 Hz, 1H), 3.77–3.67 (m, 4H), 3.11 (dd, *J* = 5.1, 3.5 Hz, 2H), 3.09–3.06 (m, 4H), 3.05–3.00 (m, 2H), 2.50–2.43 (m, 2H), 2.36 (s, 3H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 173.40, 160.90, 151.70, 151.26, 140.00, 135.89,

132.88, 131.33, 126.78, 123.70, 119.29, 117.53, 51.92, 49.97, 31.94, 30.00, 28.38, 18.01; MS (ESI)  $m/z = 350.95$  (M+H)<sup>+</sup>.

**4-(4-(*o*-Tolyl)piperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (9Y)**

The title compound was prepared by reaction of compound **3B** and 4-(*o*-tolyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 6:1) to give a white solid; yield 59.7%; mp 127.5–131.4°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.46 (s, 1H), 7.14 (s, 1H), 7.11 (d, *J* = 7.4 Hz, 1H), 7.02–6.97 (m, 1H), 6.97–6.90 (m, 1H), 3.48 (s, 4H), 3.02 (s, 4H), 2.89–2.80 (m, 2H), 2.26 (s, 3H), 1.86 (m, 4H), 1.74–1.56 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 165.36, 160.96, 150.16, 150.12, 138.16, 131.71, 131.14, 130.17, 125.60, 122.50, 120.06, 118.06, 50.39, 49.75, 31.65, 29.41, 27.76, 26.50, 26.29, 16.83; MS (ESI)  $m/z = 379.21$  (M+H)<sup>+</sup>.

**4-(4-(*m*-Tolyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (10X)**

The title compound was prepared by reaction of compound **3A** and 4-(*m*-tolyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 90:10) to give yellow crystals; yield 73%; mp 104–106°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.50 (s, 1H), 7.19 (dd, *J* = 10.0, 5.6 Hz, 1H), 6.83–6.78 (m, 2H), 6.75–6.72 (m, 1H), 3.76–3.70 (m, 4H), 3.37–3.31 (m, 4H), 3.11–3.06 (m, 2H), 3.06–3.00 (m, 2H), 2.50–2.43 (m, 2H), 2.34 (s, 3H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 173.43, 160.75, 151.69, 151.34, 140.22, 139.09, 135.77, 129.19, 121.32, 117.55, 117.35, 113.65, 49.55, 49.44, 31.87, 29.99, 28.37, 21.91; MS (ESI)  $m/z = 351.08$  (M+H)<sup>+</sup>.

**4-(4-(*m*-Tolyl)piperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (10Y)**

The title compound was prepared by reaction of compound **3B** and 4-(*m*-tolyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 5:1) to give a white solid; yield 28.3%; mp 130.6–131.8°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.47 (s, 1H), 7.11 (t, *J* = 8.0 Hz, 1H), 6.74 (s, 1H), 6.71 (s, 1H), 6.66 (d, *J* = 7.2 Hz, 1H), 3.47 (s, 4H), 3.28 (s, 4H), 3.08–3.00 (m, 2H), 2.88–2.83 (m, 2H), 2.27 (s, 3H), 1.93–1.83 (m, 2H), 1.69–1.56 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 165.44, 160.81, 150.21, 150.19, 138.37, 137.92, 131.05, 128.03, 120.15, 120.11, 116.17, 112.48, 49.23, 48.05, 31.64, 29.42, 27.70, 26.49, 26.29, 20.75; MS (ESI)  $m/z = 379.14$  (M+H)<sup>+</sup>.

**4-(4-(*p*-Tolyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (11X)**

The title compound was prepared by reaction of compound **3A** and 4-(*p*-tolyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 80:20) to give white crystals; yield 68.98%; mp 164–166°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.49 (s, 1H), 7.14–7.08 (m, 2H), 6.93 (d, *J* = 8.5 Hz, 2H), 3.77–3.73 (m, 4H), 3.33–3.28 (m, 4H), 3.10–3.05 (m, 2H), 3.04–2.99 (m, 2H), 2.49–2.43 (m, 2H), 2.29 (s, 3H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 173.33, 160.63, 151.60, 148.81, 140.22, 135.74, 130.34, 129.90,



117.48, 116.98, 50.15, 49.28, 31.84, 29.96, 28.35, 20.59; MS (ESI)  $m/z = 351.20$  (M+H)<sup>+</sup>.

**4-(4-(*p*-Tolyl)piperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (11Y)**

The title compound was prepared by reaction of compound **3B** and 4-(*p*-tolyl)piperazine according to the general procedure. The product was purified by CC (hexane/EtOAc, 8:1) to give a white solid; yield 47.3%; mp 163.2–164.8°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.47 (s, *J* = 4.7 Hz, 1H), 7.03 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 3.47 (s, 4H), 3.23 (s, 4H), 3.08–2.99 (m, 2H), 2.90–2.72 (m, 2H), 2.21 (s, 3H), 1.88–1.81 (m, 2H), 1.72–1.56 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 166.48, 161.85, 151.24, 149.09, 139.36, 132.10, 129.82, 129.75, 121.12, 116.69, 50.27, 49.53, 32.68, 30.46, 28.75, 27.53, 27.34, 20.46; MS (ESI)  $m/z = 379.21$  (M+H)<sup>+</sup>.

**1-(4-(6,7-Dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)piperazin-1-yl)ethan-1-one (12X)**

The title compound was prepared by reaction of compound **3A** and *N*-acetylpiperazine according to the general procedure. The product was purified by CC (EtOAc/TEA, 100:1) to give yellow oil; yield 80%; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.42 (s, 1H), 3.60 (dd, *J* = 6.8, 3.2 Hz, 4H), 3.55 (dd, *J* = 6.6, 2.7 Hz, 2H), 3.49 (dd, *J* = 6.4, 3.9 Hz, 2H), 3.03 (dd, *J* = 13.0, 5.8 Hz, 2H), 2.97 (dd, *J* = 7.9, 6.7 Hz, 2H), 2.43–2.32 (m, 2H), 2.04 (s, 3H); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 172.40, 168.49, 159.65, 151.23, 138.96, 135.85, 116.19, 48.63, 48.51, 45.33, 40.63, 31.22, 29.35, 27.75, 21.27; MS (ESI)  $m/z = 303.12$  (M+H)<sup>+</sup>.

**1-(4-(6,7,8,9-Tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)piperazin-1-yl)ethanone (12Y)**

The title compound was prepared by reaction of compound **3B** and *N*-acetylpiperazine according to the general procedure. The product was purified by CC (EtOAc) to give a white solid; yield 35.7%; mp 144.4–146.3°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.45 (s, 1H), 3.77–3.52 (m, 4H), 3.32 (m, 4H), 3.02–2.97 (m, 2H), 2.89–2.82 (m, 2H), 2.08 (s, 3H), 1.92–1.83 (m, 2H), 1.72–1.55 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 169.26, 166.71, 161.48, 151.13, 139.91, 131.77, 121.15, 50.89, 49.53, 45.78, 40.97, 32.62, 30.45, 28.63, 21.39; MS (ESI)  $m/z = 331.17$  (M+H)<sup>+</sup>.

**4-(4-Methylpiperazin-1-yl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (13X)**

The title compound was prepared by reaction of compound **3A** and 4-methylpiperazine according to the general procedure. The product was purified by CC (EtOAc/TEA, 100:1) to give beige crystals; yield 77%; mp 100–102°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.46 (s, 1H), 3.67–3.59 (m, 4H), 3.06–3.02 (m, 2H), 3.00 (ddd, *J* = 8.3, 3.3, 1.6 Hz, 2H), 2.63–2.59 (m, 4H), 2.47–2.41 (m, 2H), 2.39 (s, 3H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 173.40, 160.56, 151.65, 140.00, 135.81, 117.38, 54.91, 49.04, 46.15, 31.85, 29.97, 28.37; MS (ESI)  $m/z = 275.08$  (M+H)<sup>+</sup>.

**4-(4-Methylpiperazin-1-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (13Y)**

The title compound was prepared by reaction of compound **3B** and 4-methylpiperazine according to the general procedure. The product was purified by CC (1% TEA in EtOAc) to give a white solid; yield 67%; mp 115.2–116°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.44 (s, 1H), 3.36 (s, 4H), 3.04–2.90 (m, 2H), 2.84 (m, 2H), 2.51 (s, 4H), 2.29 (s, 3H), 1.93–1.77 (m, 2H), 1.73–1.53 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 166.37, 161.77, 151.19, 139.02, 132.15, 120.92, 54.66, 50.11, 46.18, 32.69, 30.44, 28.75, 27.51, 27.33; MS (ESI)  $m/z = 395.17$  (M+H)<sup>+</sup>.

***N*-Phenyl-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-amine (14X)**

The title compound was prepared by reaction of compound **3A** and aniline according to the general procedure. The product was purified by CC (hexane/EtOAc, 80:20) to give brown crystals; yield 79.3%; mp 194–196°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.51 (s, 1H), 7.71–7.62 (m, 2H), 7.40 (dd, *J* = 10.8, 5.2 Hz, 2H), 7.14 (t, *J* = 7.4 Hz, 1H), 7.00 (s, 1H), 3.13 (t, *J* = 7.2 Hz, 2H), 3.05 (dd, *J* = 10.6, 4.2 Hz, 2H), 2.60 (dt, *J* = 14.5, 7.3 Hz, 2H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 171.45, 154.50, 152.41, 140.44, 138.51, 133.91, 129.31, 124.26, 121.17, 114.21, 29.76, 29.48, 28.19; MS (ESI)  $m/z = 268.04$  (M+H)<sup>+</sup>.

***N*-Phenyl-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-amine (14Y)**

The title compound was prepared by reaction of compound **3B** and aniline according to the general procedure. The product was purified by CC (hexane/EtOAc, 8:1) to give a light brown powder; yield 47.2%; mp 153.9–155.5°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.40 (s, 1H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.09–7.00 (m, 1H), 3.12–3.02 (m, 2H), 2.92–2.80 (m, 2H), 1.81 (m, 6H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 165.37, 154.95, 152.21, 138.55, 138.53, 129.78, 129.10, 124.15, 121.64, 117.87, 30.41, 30.36, 29.07, 26.97, 26.36; MS (ESI)  $m/z = 379.21$  (M+H)<sup>+</sup>.

***N*-Benzyl-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-amine (15X)**

The title compound was prepared by reaction of compound **3A** and benzylamine according to the general procedure. The product was purified by CC (hexane/EtOAc, 90:10) to give beige powder; yield 87%; mp 155–157°C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.42 (s, 1H), 7.39–7.33 (m, 4H), 7.32–7.28 (m, 1H), 5.37 (t, *J* = 4.8 Hz, 1H), 4.82 (d, *J* = 5.6 Hz, 2H), 3.02–2.93 (m, 4H), 2.62–2.43 (m, 2H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 170.78, 156.72, 153.11, 139.06, 138.64, 134.39, 128.96, 127.73, 127.67, 113.40, 44.82, 29.63, 29.34, 28.10; MS (ESI)  $m/z = 282.094$  (M+H)<sup>+</sup>.

***N*-Benzyl-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-amine (15Y)**

The title compound was prepared by reaction of compound **3B** and benzylamine according to the general procedure. The product was purified by CC (hexane/EtOAc, 4.5:1) to give white crystals; yield 53.9%; mp 98.5–100.7°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.41 (s, 1H),



7.38–7.27 (m, 5H), 5.58 (s, 1H), 4.82 (d,  $J = 5.4$  Hz, 2H), 2.97 (m, 2H), 2.89 (m, 2H), 1.82 (m, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  164.35, 157.14, 152.65, 138.69, 137.17, 130.14, 128.83, 127.66, 127.55, 117.19, 45.07, 30.34, 30.30, 28.88, 27.03, 26.25; MS (ESI)  $m/z = 310.15$  (M+H) $^+$ .

*N*-(2-Cyclohexylethyl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]-thieno[2,3-*d*]pyrimidin-4-amine (16Y)

The title compound was prepared by reaction of compound **3B** and 2-cyclohexylethylamine according to the general procedure. The product was purified by CC (hexane/EtOAc, 5:1) to give a yellow solid; yield 50.67%; mp 113.4–115.6°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.32 (s, 1H), 5.17 (s, 1H), 3.56–3.47 (m, 2H), 2.97–2.88 (m, 2H), 2.83–2.76 (m, 2H), 1.88–1.43 (m, 14H), 1.37–1.02 (m, 5H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  163.90, 157.37, 152.67, 136.79, 130.20, 117.10, 39.11, 36.93, 35.78, 33.30, 30.42, 30.29, 28.87, 27.07, 26.50, 26.29, 26.27; MS (ESI)  $m/z = 330.20$  (M+H) $^+$ .

*N*-(Cyclohexylmethyl)-6,7-dihydro-5H-cyclopenta[4,5]-thieno[2,3-*d*]pyrimidin-4-amine (17X)

The title compound was prepared by reaction of compound **3A** and cyclohexylmethylamine according to the general procedure. The product was purified by CC (hexane/EtOAc, 70:30) to give white powder; yield 100%; mp 122–124°C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.37 (s, 1H), 5.14 (s, 1H), 3.43 (dd,  $J = 6.8, 5.8$  Hz, 2H), 3.05–2.92 (m, 4H), 2.58–2.49 (m, 2H), 1.83–1.72 (m, 4H), 1.71–1.57 (m, 2H), 1.31–1.11 (m, 3H), 1.01 (qd,  $J = 12.0, 2.7$  Hz, 2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  170.64, 157.17, 153.30, 138.57, 134.39, 113.41, 47.01, 37.94, 31.07, 29.62, 29.40, 28.12, 26.57, 25.98; MS (ESI)  $m/z = 288.74$  (M+H) $^+$ .

#### 4.1.7 | General procedure for the synthesis of hydrazones

Compound **4A** or **4B** (0.2 g, 0.85 mmol) and the respective aldehyde (1.18 equiv, 1 mmol) were dissolved in EtOH (10–15 mL). The mixture was refluxed for 24 h. After cooling to room temperature, ethanol was removed in vacuum and silica gel flash CC was done on most of the compounds followed by washing of the compounds with 81% EtOH to obtain compounds **18X–26X** and **18Y–26Y**, respectively.<sup>[30]</sup>

(*E*)-4-(2-(*o*-Bromobenzylidene)hydrazinyl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (18X)

The title compound was prepared by reaction of compound **4A** and *o*-bromobenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 70:30) to give orange crystals; yield 75.5%; mp 199–201°C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.42 (d,  $J = 7.5$  Hz, 2H), 8.06 (d,  $J = 7.7$  Hz, 1H), 7.59–7.50 (m, 1H), 7.35–7.26 (m, 1H), 7.20 (td,  $J = 8.0, 1.7$  Hz, 1H), 3.15 (dd,  $J = 17.1, 10.2$  Hz, 2H), 3.04–2.96 (m, 2H), 2.55–2.42 (m, 2H); MS (ESI)  $m/z = 372.90$  (M+H) $^+$ .

(*E*)-4-(2-(*o*-Bromobenzylidene)hydrazinyl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (18Y)

The title compound was prepared by reaction of compound **4B** and *o*-bromobenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 3:1) to give orange solid; yield 33.8%; mp 180.5–183.1°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  10.45 (s, 1H), 8.76 (s, 1H), 7.97 (d,  $J = 7.7$  Hz, 1H), 7.63 (s, 1H), 7.51 (d,  $J = 7.9$  Hz, 1H), 7.25 (t,  $J = 7.5$  Hz, 1H), 7.18–7.11 (m, 1H), 3.43 (m, 2H), 2.79 (m, 2H), 1.83 (m, 2H), 1.63–1.53 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  153.01, 140.71, 138.63, 135.87, 134.20, 133.41, 132.06, 130.94, 129.95, 128.06, 127.34, 124.93, 124.58, 32.63, 30.06, 28.47, 27.73, 27.28; MS (ESI)  $m/z = 402.76$  (M+H) $^+$ .

(*E,Z*)-4-(2-(*m*-Bromobenzylidene)hydrazinyl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (19X)

The title compound was prepared by reaction of compound **4A** and *m*-bromobenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 50:50) to give yellow powder; yield 87.73%; mp 193–195°C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  12.20–11.09 (m, 1H), 8.47–8.24 (m, 2H), 7.90–7.80 (m, 1H), 7.68–7.52 (m, 2H), 7.40 (dt,  $J = 13.8, 7.8$  Hz, 1H), 3.12 (t,  $J = 7.2$  Hz, 1H), 2.95–3 (m, 3H), 2.42–2.30 (m, 2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ )  $\delta$  173.21, 162.83, 154.72, 151.57, 151.06, 149.05, 143.63, 143.26, 139.59, 138.46, 137.93, 136.83, 136.71, 132.05, 131.86, 131.00, 130.61, 129.29, 129.11, 127.22, 126.00, 122.20, 122.15, 116.61, 112.26, 32.38, 29.67, 29.48, 29.09, 27.46, 27.05; MS (ESI)  $m/z = 373.06$  (M+H) $^+$ .

(*E*)-4-(2-(*m*-Bromobenzylidene)hydrazinyl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (19Y)

The title compound was prepared by reaction of compound **4B** and *m*-bromobenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 3:1) to give a brown solid; yield 28.3%; mp 214.8–217.8°C;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.98 (s, 1H), 8.41 (s, 1H), 8.31–8.29 (m, 1H), 7.87 (d,  $J = 7.7$  Hz, 1H), 7.83 (d,  $J = 3.7$  Hz, 1H), 7.59 (ddd,  $J = 8.0, 1.9, 0.9$  Hz, 1H), 7.40 (t,  $J = 7.8$  Hz, 1H), 3.52–3.42 (m, 2H), 2.92–2.77 (m, 2H), 1.86 (m, 2H), 1.70–1.54 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  156.78, 151.86, 150.47, 143.92, 138.38, 137.44, 137.15, 132.61, 131.11, 129.84, 127.81, 122.69, 119.73, 32.57, 29.68, 28.33, 27.80, 27.36; MS (ESI)  $m/z = 400.97$  (M+H) $^+$ .

(*E,Z*)-4-(2-(*p*-Bromobenzylidene)hydrazinyl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (20X)

The title compound was prepared by reaction of compound **4A** and *p*-bromobenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 50:50) to give yellow powder; yield 80%; mp 205–209°C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  12.05–11.08 (m, 1H), 8.44–8.22 (m, 2H), 7.92–7.79 (m, 1H), 7.66–7.59 (m, 3H), 3.10 (t,  $J = 7.2$  Hz, 1H), 2.96 (dd,  $J = 15.6, 8.2$  Hz, 2H), 2.90 (t,  $J = 7.2$  Hz, 1H), 2.35 (tt,  $J = 14.7, 7.4$  Hz,



2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ )  $\delta$  173.17, 162.71, 154.78, 151.57, 151.43, 148.87, 143.87, 143.68, 139.60, 138.34, 137.86, 136.82, 134.75, 133.65, 131.84, 131.47, 129.52, 128.82, 122.86, 122.59, 116.64, 112.27, 32.32, 29.67, 29.47, 29.08, 27.46, 27.09; MS (ESI)  $m/z$  = 373.08 ( $\text{M}+\text{H}$ ) $^+$ .

(E)-4-(2-(*p*-Bromobenzylidene)hydrazinyl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (20Y)

The title compound was prepared by reaction of compound 4B and *p*-bromobenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 4:1) to give an off-white solid: yield 76.09%; mp 230.3–232.5°C;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.93 (d,  $J$  = 2.9 Hz, 1H), 8.42 (s, 1H), 7.93 (d,  $J$  = 8.5 Hz, 2H), 7.82 (d,  $J$  = 3.6 Hz, 1H), 7.65 (d,  $J$  = 8.4 Hz, 2H), 3.47 (m, 2H), 2.87–2.77 (m, 2H), 1.86 (m, 2H), 1.74–1.39 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  156.65, 152.22, 150.28, 143.96, 137.37, 137.15, 135.20, 131.96, 130.08, 123.43, 119.75, 32.58, 29.67, 28.32, 27.80, 27.36; MS (ESI)  $m/z$  = 400.99

( $\text{M}+\text{H}$ ) $^+$ .

(E,Z)-4-(2-(2-Fluorobenzylidene)hydrazinyl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (21X)

The title compound was prepared by reaction of compound 4A and *o*-fluorobenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 60:40) to give yellow powder: yield 80%; mp 190–192°C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.64–11.55 (m, 1H), 8.50 (d,  $J$  = 15.2 Hz, 1H), 8.43–8.32 (m, 1H), 7.92–7.79 (m, 1H), 7.49–7.39 (m, 1H), 7.33–7.22 (m, 2H), 3.11 (t,  $J$  = 7.2 Hz, 1H), 2.93 (dt,  $J$  = 13.6, 7.3 Hz, 3H), 2.40–2.28 (m, 2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ )  $\delta$  173.25, 160.59 (d,  $J_{\text{CF}}$  = 249.3 Hz), 154.68, 151.63, 149.18, 144.69 (d,  $J_{\text{CF}}$  = 3.1 Hz), 143.72, 139.72, 138.48, 138.02, 137.58 (d,  $J_{\text{CF}}$  = 4.7 Hz), 136.90, 131.56 (d,  $J_{\text{CF}}$  = 9.2 Hz), 131.37 (d,  $J_{\text{CF}}$  = 8.6 Hz), 127.45 (d,  $J_{\text{CF}}$  = 1.9 Hz), 126.62 (d,  $J_{\text{CF}}$  = 2.0 Hz), 124.97 (d,  $J_{\text{CF}}$  = 3.2 Hz), 124.54, 122.95, 122.87, 122.14, 122.06, 116.65, 116.04 (d,  $J_{\text{CF}}$  = 20.6 Hz), 115.80, 112.33, 32.41, 29.71, 29.54, 29.16, 27.52, 27.14; MS (ESI)  $m/z$  = 313.13 ( $\text{M}+\text{H}$ ) $^+$ .

(E)-4-(2-(*o*-Fluorobenzylidene)hydrazinyl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (21Y)

The title compound was prepared by reaction of compound 4B and *o*-fluorobenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 4:1) to give a light brown solid: yield 32.37%; mp 173.7–178.3°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.63 (s, 1H), 7.97 (td,  $J$  = 7.6, 1.5 Hz, 2H), 7.27 (m, 1H), 7.05 (m, 2H), 3.39 (m, 2H), 2.84–2.72 (m, 2H), 1.88–1.80 (m, 2H), 1.69–1.61 (m, 4H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  162.78, 160.91, 159.70 (d,  $J_{\text{CF}}$  = 244.2 Hz), 156.79, 143.67, 135.50, 133.08 (d,  $J_{\text{CF}}$  = 8.3 Hz), 132.05 (d,  $J_{\text{CF}}$  = 9.5 Hz), 131.30 (d,  $J_{\text{CF}}$  = 8.5 Hz), 127.30, 124.18 (d,  $J_{\text{CF}}$  = 3.5 Hz), 116.44 (d,  $J_{\text{CF}}$  = 20.7 Hz), 115.94 (d,  $J_{\text{CF}}$  = 21.1 Hz), 30.52, 29.68, 28.90, 27.81, 27.29; MS (ESI)  $m/z$  = 341.1 ( $\text{M}+\text{H}$ ) $^+$ .

(E)-4-(2-(5-Bromo-2-methoxybenzylidene)hydrazinyl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (22X)

The title compound was prepared by reaction of compound 4A and 5-bromo-2-methoxybenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (DCM/MeOH, 100:0.5) to give an orange powder: yield 77%; mp 258–260°C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.63–11.52 (m, 1H), 8.55–8.48 (m, 2H), 7.92–7.84 (m, 1H), 7.58–7.50 (m, 1H), 7.13–7.02 (m, 1H), 3.87 (d,  $J$  = 2.8 Hz, 3H), 3.15–3.04 (m, 1H), 3.02–2.89 (m, 3H), 2.36 (dt,  $J$  = 11.7, 4.9 Hz, 2H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ )  $\delta$  156.58, 154.64, 151.67, 143.67, 140.82, 138.86, 132.93, 127.99, 127.80, 127.61, 114.27, 114.14, 113.57, 56.07, 29.49, 27.34, 27.05; MS (ESI)  $m/z$  = 402.98 ( $\text{M}+\text{H}$ ) $^+$ .

(E)-4-(2-(5-Bromo-2-methoxybenzylidene)hydrazinyl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (22Y)

The title compound was prepared by reaction of compound 4B and 5-bromo-2-methoxybenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 5:1) to give an yellow solid: yield 16%; mp 237.2–238.5°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  10.50 (s, 1H), 8.70 (s, 1H), 8.05 (s, 1H), 7.65 (s, 1H), 7.35 (dd,  $J$  = 8.8, 2.5 Hz, 1H), 6.75 (t,  $J$  = 14.2 Hz, 1H), 3.80 (s, 3H), 3.41 (m, 2H), 2.78 (m, 2H), 1.83 (m, 2H), 1.67 (m, 4H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  157.27, 156.04, 148.50, 143.22, 140.90, 138.39, 137.19, 135.52, 133.35, 128.94, 125.79, 120.25, 112.99, 55.82, 32.36, 29.97, 29.68, 27.64, 27.20; MS (ESI)  $m/z$  = 430.98 ( $\text{M}+\text{H}$ ) $^+$ .

(E)-4-(2-(*o*-Fluoro-*p*-methoxybenzylidene)hydrazinyl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (23X)

The title compound was prepared by reaction of compound 4A and *o*-fluoro-4-methoxybenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 70:30) to give a brown powder: yield 87%; mp 207–209°C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.60–11.51 (m, 1H), 8.40 (d,  $J$  = 5.5 Hz, 2H), 7.79 (s, 1H), 6.90 (t,  $J$  = 11.1 Hz, 2H), 3.82 (s, 3H), 3.36 (s, 1H), 3.11 (s, 1H), 2.95 (s, 2H), 2.38–2.28 (m, 2H); MS (ESI)  $m/z$  = 343.15 ( $\text{M}+\text{H}$ ) $^+$ .

(E)-4-(2-(*o*-Fluoro-*p*-methoxybenzylidene)hydrazinyl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (23Y)

The title compound was prepared by reaction of compound 4B and *o*-fluoro-*p*-methoxybenzaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 4:1) to give an off-white solid: yield 73%; mp 230.3–235.8°C;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.86 (d,  $J$  = 2.5 Hz, 1H), 8.46 (s, 1H), 8.30 (dd,  $J$  = 11.3, 6.6 Hz, 1H), 7.80 (d,  $J$  = 3.7 Hz, 1H), 6.96–6.90 (m, 1H), 6.90–6.87 (m, 1H), 3.84 (s, 3H), 3.50–3.43 (m, 2H), 2.87–2.76 (m, 2H), 1.85 (m, 2H), 1.61 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  162.65, 162.50, 162.30 (d,  $J_{\text{CF}}$  = 253.1 Hz), 156.36, 149.88, 145.50 (d,  $J_{\text{CF}}$  = 3.2 Hz), 143.97, 137.21, 128.79 (d,  $J_{\text{CF}}$  = 4 Hz), 119.70, 115.68 (d,  $J_{\text{CF}}$  = 10.4 Hz), 112.01, 101.60 (d,  $J_{\text{CF}}$  = 25.1 Hz), 56.37, 32.57, 29.65, 28.31, 27.82, 27.33; MS (ESI)  $m/z$  = 371.1 ( $\text{M}+\text{H}$ ) $^+$ .



**(E,Z)-4-(2-(Naphthalen-1-ylmethylene)hydrazinyl)-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-d]pyrimidine (24X)**

The title compound was prepared by reaction of compound **4A** and naphthaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (DCM/MeOH, 100:1) to give an orange powder: yield 80%; mp 210–212°C;  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.01–11.20 (m, 1H), 8.54–8.31 (m, 2H), 8.16–8.08 (m, 1H), 8.03–7.78 (m, 4H), 7.58–7.53 (m, 2H), 3.21 (t,  $J$  = 7.2 Hz, 1H), 2.99 (t,  $J$  = 7.3 Hz, 2H), 2.92 (t,  $J$  = 7.2 Hz, 1H), 2.42–2.33 (m, 2H);  $^{13}\text{C}$  NMR (126 MHz, DMSO- $d_6$ )  $\delta$  173.55, 173.17, 162.60, 154.84, 152.75, 151.64, 148.70, 145.27, 143.78, 139.67, 138.24, 137.84, 136.94, 133.70, 133.36, 133.30, 132.96, 132.11, 128.88, 128.51, 128.27, 128.19, 128.14, 127.97, 127.79, 127.74, 126.95, 126.83, 126.77, 126.63, 123.94, 123.00, 116.72, 112.27, 32.43, 29.72, 29.51, 29.11, 27.50, 27.12; MS (ESI)  $m/z$  = 345.17 (M+H) $^+$ .

**(E)-4-(2-(Naphthalen-1-ylmethylene)hydrazinyl)-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-d]pyrimidine (24Y)**

The title compound was prepared by reaction of compound **4B** and naphthaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 3:1) to give white solid: yield 67%; mp 185.7–187.1°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  10.47 (s, 1H), 9.17 (s, 1H), 8.53 (d,  $J$  = 8.3 Hz, 1H), 7.99 (d,  $J$  = 7.2 Hz, 1H), 7.83 (s, 1H), 7.81 (s, 1H), 7.62 (s, 1H), 7.52 (d,  $J$  = 6.8 Hz, 1H), 7.47 (dd,  $J$  = 4.8, 2.8 Hz, 1H), 7.43 (dd,  $J$  = 7.2, 3.2 Hz, 1H), 3.61–3.49 (m, 2H), 2.93–2.78 (m, 2H), 1.93 (m, 2H), 1.75 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  155.91, 153.09, 149.90, 140.93, 138.47, 137.21, 133.92, 131.30, 130.98, 130.42, 128.83, 126.87, 126.64, 126.06, 125.39, 123.88, 120.27, 32.68, 30.09, 28.54, 27.75, 27.34; MS (ESI)  $m/z$  = 402.76 (M+H) $^+$ .

**(E)-2-((2-(6,7-Dihydro-5H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazono)methyl)naphthalen-1-ol (25X)**

The title compound was prepared by reaction of compound **4A** and 1-hydroxy-2-naphthaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 70:30) to give a yellow powder: yield 72%; mp 266–268°C;  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.88 (s, 1H), 10.54 (s, 1H), 8.75 (s, 1H), 8.55 (s, 1H), 8.32 (d,  $J$  = 7.2 Hz, 1H), 7.86 (d,  $J$  = 8.5 Hz, 1H), 7.57 (dd,  $J$  = 5.5, 4.2 Hz, 1H), 7.55 (d,  $J$  = 7.5 Hz, 1H), 7.53–7.49 (m, 1H), 7.43 (d,  $J$  = 8.5 Hz, 1H), 3.19 (s, 2H), 2.97 (s, 2H), 2.44 (d,  $J$  = 5.9 Hz, 2H);  $^{13}\text{C}$  NMR (126 MHz, DMSO- $d_6$ )  $\delta$  172.57, 157.34, 155.13, 153.55, 153.36, 148.60, 139.69, 136.19, 135.09, 128.45, 128.36, 127.10, 126.45, 125.32, 123.36, 119.62, 112.85, 30.21, 30.14, 28.15; MS (ESI)  $m/z$  = 361.16 (M+H) $^+$ .

**(E)-2-((2-(6,7,8,9-Tetrahydro-5H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazono)methyl)naphthalen-1-ol (25Y)**

The title compound was prepared by reaction of compound **4B** and 1-hydroxy-2-naphthaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 2.5:1) to give brown solid: yield 33%; mp 233.2–234.9°C;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  11.81 (s, 1H), 11.45 (s, 1H), 8.80 (s, 1H), 8.31 (dd,  $J$  = 6.6, 2.9 Hz, 1H), 7.88 (dd,  $J$  = 6.4, 2.9 Hz, 1H), 7.85 (d,  $J$  = 4.4 Hz, 1H), 7.75–7.68 (m, 1H), 7.62–7.57 (m, 1H), 7.57–7.53 (m, 1H), 7.45 (d,

$J$  = 8.5 Hz, 1H), 3.52 (m, 2H), 2.85 (m, 2H), 1.87 (m, 2H), 1.65 (m, 4H);  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  156.86, 156.17, 154.44, 147.82, 144.05, 137.41, 137.15, 135.00, 128.15, 128.03, 127.60, 126.09, 124.76, 122.95, 119.65, 119.42, 113.51, 32.53, 29.63, 28.40, 27.75, 27.33; MS (ESI)  $m/z$  = 389.10 (M+H) $^+$ .

**(E)-6-((2-(6,7-Dihydro-5H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazono)methyl)naphthalen-2-ol (26X)**

The title compound was prepared by reaction of compound **4A** and 6-hydroxy-2-naphthaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (hexane/EtOAc, 70:30) to give a green powder: yield 38%; mp 299–301°C;  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  10.03 (s, 1H), 8.65 (s, 1H), 8.39 (s, 1H), 8.07 (s, 2H), 7.84 (d,  $J$  = 8.8 Hz, 1H), 7.75 (d,  $J$  = 9.0 Hz, 1H), 7.20–7.10 (m, 2H), 3.19 (t,  $J$  = 6.8 Hz, 2H), 2.99 (t,  $J$  = 7.1 Hz, 2H), 2.47–2.37 (m, 2H);  $^{13}\text{C}$  NMR (126 MHz, DMSO- $d_6$ )  $\delta$  157.00, 151.26, 148.86, 145.65, 137.35, 135.95, 130.30, 130.12, 130.10, 128.23, 127.24, 126.75, 123.50, 119.34, 114.35, 110.31, 109.16, 30.28, 29.47, 27.45; MS (ESI)  $m/z$  = 361.15 (M+H) $^+$ .

**(E)-6-((2-(6,7,8,9-Tetrahydro-5H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazono)methyl)naphthalen-2-ol (26Y)**

The title compound was prepared by reaction of compound **4B** and 6-hydroxy-2-naphthaldehyde according to the general procedure for hydrazone synthesis. The product was purified by CC (5% acetone in DCM) to give brown solid: yield 33%; mp 232.3–237°C;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  11.92 (s, 1H),  $\delta$  10.01 (s, 1H), 8.54 (d,  $J$  = 13.7 Hz, 1H), 8.28 (d,  $J$  = 8.6 Hz, 1H), 8.14 (s, 1H), 7.86 (s, 1H), 7.81 (d,  $J$  = 10.8 Hz, 1H), 7.77 (s, 1H), 7.22 (d,  $J$  = 2.2 Hz, 1H), 7.17 (dd,  $J$  = 8.7, 2.4 Hz, 1H), 3.55 (m, 2H), 2.87 (m, 2H), 1.92 (m, 2H), 1.68 (m, 4H);  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  156.80, 156.13, 153.88, 149.56, 144.02, 137.15, 137.11, 135.98, 130.57, 130.40, 129.53, 127.84, 126.72, 124.68, 119.78, 119.46, 109.58,  $\delta$  32.56, 29.64, 28.30, 27.80, 27.37; MS (ESI)  $m/z$  = 389.14 (M+H) $^+$ .

**4.2 | PDE assay**

Purified PDE5, 7 and 9 (BPS Biosciences) was added to the wells of black 96-well non-binding plates at concentrations required to exhibit 80% activity. Immediately, the protein was treated with compound or vehicle control and allowed to incubate for 30 min at 37°C. Following this incubation, 25 nM FL-cGMP (Molecular Devices) was added to each assay well and allowed to incubate for 1.5 h at 30°C. After incubation, IMAP FP Phosphodiesterase Evaluation Assay (Molecular Devices) binding reagent was added to each well and the plates were incubated for an additional 10 min at 30°C. FP was measured according to manufacturer's specifications using a Biotek Synergy 4 plate reader. All synthesized compounds were tested for their ability to inhibit recombinant PDE5 and PDE9 using cGMP as a substrate and PDE7 using cAMP as a substrate, respectively. For enzyme assays, the  $\text{IC}_{50}$  value was determined by testing a range of four concentrations with at least two replicates per concentration, which was repeated at least twice to confirm reproducibility of  $\text{IC}_{50}$  values and extrapolation of the curve. Concentration-dependent curves were analyzed using



Prism™ 4 software (GraphPad) to calculate IC<sub>50</sub> values using a four parameter logistic equation.

### 4.3 | Molecular modeling

Molecular docking of the most active compound on PDE5, compound 15Y, was implemented on PDE5 active site using MOE 2010. First, construction of the compound was carried out on MOE window followed by minimization of the compound. Then 2H42 code, PDE5 co-crystallized with sildenafil, was imported into MOE window and the structure was protonated. Following this, isolation of the pocket was carried out and docking of the compound was carried out after removal of the co-crystallized ligand from the pocket. The poses from the ligand conformation were generated using triangle matcher, the scoring function used was ASE with alpha triangle refinement. The pose with a high score showing essential features required for PDE5 activity was chosen. Self-docking was carried out for the co-crystallized ligand, sildenafil, using the same docking procedure settings to ensure a more accurate docking process.


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### CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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### SUPPORTING INFORMATION

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